

## CLAIMS

1. An electrochemical sensor strip, comprising:  
a base;  
a first electrode on the base;  
5 a second electrode on the base;  
an oxidoreductase enzyme and a mediator on the first electrode;  
and  
a soluble redox species on the second electrode.
2. The electrochemical sensor strip of claim 1, wherein the soluble  
10 redox species comprises a member selected from the group consisting of an  
electroactive organic molecule, an organotransition metal complex, a  
transition metal coordination complex, and mixtures thereof.
3. The electrochemical sensor strip of claim 1, wherein the soluble  
redox species comprises ferrocyanide or ferricyanide.
- 15 4. The electrochemical sensor strip of claim 1, wherein the soluble  
redox species comprises ruthenium(II)hexaamine or ruthenium(III)hexaamine.
5. The electrochemical sensor strip of claim 1, wherein the soluble  
redox species comprises an electroactive organic molecule selected from the  
group consisting of coenzyme pyrroloquinoline quinone (PQQ), substituted  
20 benzoquinones, substituted naphthoquinones, N-oxides, nitroso compounds,  
hydroxylamines, oxines, flavins, phenazines, phenothiazines, indophenols,  
indamines, phenazinium salts, phenoxazinium salts, and mixtures thereof.
6. The electrochemical sensor strip of claim 1, further comprising a  
second redox species on the second electrode; wherein the soluble redox  
25 species is a first redox species of a redox pair comprising the first species and  
the second species; and wherein the molar ratio of the first redox species to  
the second redox species is greater than about 1.2:1.

7. The electrochemical sensor strip of claim 6, wherein the molar ratio of the first redox species to the second redox species is greater than about 2:1.
8. The electrochemical sensor strip of claim 6, wherein the molar ratio of the first redox species to the second redox species is greater than about 10:1.
9. The electrochemical sensor strip of claim 6, wherein the second redox species is present in an amount less than about 1 part per thousand.
10. The electrochemical sensor strip of claim 6, wherein the first redox species has a standard reduction potential of +0.24 volts or greater.
11. The electrochemical sensor strip of claim 6, wherein the first redox species has a reduction potential of about +0.35 volts or greater.
12. The electrochemical sensor strip of claim 6, further comprising the second redox species on the first electrode.
13. The electrochemical sensor strip of claim 1, wherein the mediator comprises an electroactive organic molecule selected from the group consisting of coenzyme pyrroloquinoline quinone (PQQ), substituted benzoquinones, substituted naphthoquinones, N-oxides, nitroso compounds, hydroxylamines, oxines, flavins, phenazines, phenothiazines, indophenols, indamines, phenazinium salts, phenoxazinium salts, and mixtures thereof.
14. The electrochemical sensor strip of claim 1, wherein the mediator comprises 3-phenylimino-3H-phenothiazine.
15. The electrochemical sensor strip of claim 1, wherein the mediator comprises 3-phenylimino-3H-phenoxazine.
16. The electrochemical sensor strip of claim 1, wherein the oxidoreductase is selected from the group consisting of glucose dehydrogenase, glucose oxidase, cholesterol esterase, cholesterol oxidase, lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, lactate

oxidase, lactate dehydrogenase, diaphorase, pyruvate oxidase, alcohol oxidase, bilirubin oxidase, uricase, glutathione reductase, and carbon monoxide oxidoreductase.

5           17.    The electrochemical sensor strip of claim 1, wherein the oxidoreductase comprises an enzyme selected from the group consisting of an oxidase and a dehydrogenase; and wherein the soluble redox species is a reducible species.

          18.    The electrochemical sensor strip of claim 17, wherein the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

10           19.    The electrochemical sensor strip of claim 1, wherein the oxidoreductase comprises a reductase; and wherein the soluble redox species is an oxidizable species.

          20.    The electrochemical sensor strip of claim 1, further comprising a lid.

15           21.    The electrochemical sensor strip of claim 1, wherein the second electrode comprises a surface layer comprising a non-ionizing conducting material.

          22.    The electrochemical sensor strip of claim 21, wherein the surface layer comprises carbon.

20           23.    The electrochemical sensor strip of claim 1, wherein the second electrode comprises a non-ionizing conducting material.

          24.    The electrochemical sensor strip of claim 1, further comprising a third electrode on the base; and a second soluble redox species on the third electrode.

25           25.    The electrochemical sensor strip of claim 24, wherein the second soluble redox species is substantially identical to the soluble redox species on the second electrode.

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26. An electrochemical sensor strip, comprising:  
a base;  
a first electrode on the base;  
a second electrode on the base;  
5 an enzyme on the first electrode, the enzyme selected from the group consisting of glucose oxidase, glucose dehydrogenase, and mixtures thereof;  
a mediator on the first electrode; and  
a soluble redox species on the second electrode;  
10 wherein the soluble redox species is a reducible species selected from the group consisting of an organotransition metal complex, a transition metal coordination complex, and mixtures thereof.
27. The electrochemical sensor strip of claim 26, wherein the mediator is selected from the group consisting of 3-phenylimino-3H-phenothiazine, 3-phenylimino-3H-phenoxazine, and mixtures thereof.  
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28. The electrochemical sensor strip of claim 26, wherein the soluble redox species is selected from the group consisting of ferricyanide and ruthenium(III)hexaamine.
29. The electrochemical sensor strip of claim 26, further comprising  
20 a third electrode on the base; and a second soluble redox species on the third electrode.
30. The electrochemical sensor strip of claim 29, wherein the second soluble redox species is substantially identical to the soluble redox species on the second electrode.  
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31. A method of making an electrochemical sensor strip, comprising:  
depositing a first electrode on a base;  
depositing a second electrode on the base;

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applying a first layer onto the first electrode, the first layer comprising an oxidoreductase and a mediator; and

applying a second layer onto the second electrode, the second layer comprising a soluble redox species.

5           32.    The method of claim 31, wherein the oxidoreductase comprises an enzyme selected from the group consisting of an oxidase and a dehydrogenase; and wherein the soluble redox species is a reducible species.

          33.    The method of claim 32, wherein the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

10          34.    The method of claim 31, wherein the oxidoreductase comprises a reductase; and wherein the soluble redox species is an oxidizable species.

          35.    The method of claim 31, wherein the second layer further comprises a second redox species; wherein the soluble redox species is a first redox species of a redox pair comprising the first species and the second species; and wherein the molar ratio of the first redox species to the second redox species is greater than about 10:1.

          36.    The method of claim 35, wherein the second redox species is present in the second layer in an amount less than about 1 part per thousand.

20          37.    The method of claim 35, wherein the first redox species has a standard reduction potential of +0.24 volts or greater.

          38.    The method of claim 35, wherein the first redox species has a reduction potential of about +0.35 volts or greater.

          39.    The method of claim 31, wherein the depositing the first electrode comprises screen printing a pattern of conductive carbon.

25          40.    The method of claim 31, wherein the depositing the second electrode comprises screen printing a pattern of conductive carbon.

41. The method of claim 31, wherein the depositing the second electrode comprises depositing a pattern of a non-ionizing conductive material.

5 42. The method of claim 31, further comprising covering a portion of the base with a dielectric layer such that the first and second layers are exposed.

43. The method of claim 31, further comprising mating a lid to the base such that the lid is over the first and second electrodes and the first and second layers.

10 44. The method of claim 31, wherein the applying the first layer comprises dispensing a first aqueous composition comprising the oxidoreductase and the mediator.

45. The method of claim 44, wherein the first aqueous composition further comprises a redox cofactor for the oxidoreductase.

15 46. The method of claim 44, wherein the first aqueous composition further comprises a binder.

47. The method of claim 44, wherein the first aqueous composition further comprises a buffer.

20 48. The method of claim 31, wherein the applying the second layer comprises dispensing a second aqueous composition comprising the soluble redox species.

49. The method of claim 48, wherein the second aqueous composition further comprises a buffer.

25 50. The method of claim 31, further comprising depositing a third electrode on the base; and applying a third layer onto the third electrode, the third layer comprising a second soluble redox species.



51. The method of claim 50, wherein the second soluble redox species is substantially identical to the soluble redox species of the second layer.

52. The method of claim 51, wherein the composition of the third  
5 layer is substantially identical to the composition of the second layer.

53. A method of quantifying an analyte in a sample, comprising:  
contacting the sample with an electrochemical sensor strip;  
the electrochemical sensor strip comprising a first electrode and a first  
layer on the first electrode, the first layer comprising an oxidoreductase  
10 enzyme and a mediator;  
the electrochemical sensor strip also comprising a second  
electrode and a second layer on the second electrode, the second  
layer comprising a soluble redox species;  
applying an electrical potential between the first and second  
15 electrodes;  
measuring a current passing through the first and second  
electrodes and the sample; and  
correlating the current to a concentration of the analyte.

54. The method of claim 53, wherein the soluble redox species  
20 solubilizes in the sample and mixes with the enzyme and the mediator.

55. The method of claim 53, wherein the analyte is a substrate for  
the oxidoreductase enzyme.

56. The method of claim 53, wherein the analyte is a cofactor for the  
oxidoreductase enzyme and a substrate for the enzyme.

25 57. The method of claim 53, wherein the oxidoreductase comprises  
an enzyme selected from the group consisting of an oxidase and a  
dehydrogenase; and wherein the soluble redox species is a reducible species.

58. The method of claim 57, wherein the soluble redox species is selected from the group consisting of ferricyanide and ruthenium(III)hexaamine.

59. The method of claim 53, wherein the oxidoreductase comprises a reductase; and wherein the soluble redox species is an oxidizable species.

60. The method of claim 53, wherein the analyte comprises glucose, and the oxidoreductase comprises glucose oxidase or glucose dehydrogenase.

61. The method of claim 53, wherein the mediator is selected from the group consisting of 3-phenylimino-3H-phenothiazine, 3-phenylimino-3H-phenoxazine, and mixtures thereof.

62. The method of claim 53, wherein the electrochemical sensor strip further comprises a third electrode comprising the soluble redox species.

63. The method of claim 62, wherein the third electrode measures a second electrical potential between the third electrode and the first electrode; and the measured second electrical potential is used to adjust the electrical potential between the first and second electrodes.